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(54) Title: METHOD FOR THE SIMULTANEOUS DETECTION OF DIFFERENT ANTIBODIES AND/OR ANTIGENS

(57) Abstract

The present invention relates to a method for the simultaneous detection of more than one antibody and/or more than one class of antibodies or more than one antigen, or of one or more antibodies and/or one or more classes of antibodies and one or more antigens present in a test sample, use of the method for the diagnosis of or screening for disorders or diseases caused by viruses, bacteria, fungi, parasites, toxins or allergens as well as to kits for performing the method. In accordance with the present method, complexes formed between antibodies in the sample to be tested and selected optionally labelled antigens (antibody-antigen complexes), and/or between antigens in the sample to be tested and selected optionally labelled antibodies (antibody-antigen complexes) are captured by means of a substance having ability to bind antibody-antigen complexes, which substance is or will be immobilised onto a solid support. The captured labelled antibody-antigen complexes are observed or measured on the solid support and the observation or measurement is related to the presence of antibodies and/or antigens in said test sample.

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Method for the simultaneous detecti n f different antibodies and/or antigens

The present invention relates to a method for the simultaneous detection of more than one antibody and/or more than one class of antibodies or more than one antigen, or of one or more antibodies and/or one or more classes of antibodies and one or more antigens present in a test sample, use of the method for the diagnosis of or screening for disorders or diseases caused by viruses, bacteria, fungi, parasites, toxins or allergens as well as to kits for performing the method.

In accordance with the present method, complexes formed between antibodies in the sample to be tested and selected optionally labelled antigens (antibody-antigen complexes), and/or between antigens in the sample to be tested and selected optionally labelled antibodies (antibody-antigen complexes) are captured by means of a substance having ability to bind antibody-antigen complexes, which substance is or will be immobilised onto a solid support. The captured labelled antibody-antigen complexes is observed or measured on the solid support and the observation or measurement is related to the presence of antibodies and/or antigens in said test sample.

BACKGROUND OF THE INVENTION

Substances having ability to bind antibody-antigen complexes are substances that have the property of binding to antibodies that are part of complexes formed between antibodies and antigens, but that essentially do not bind to non-complexed antibodies. Complexes of antibodies and antigens (also called immune complexes) are formed *in vivo* as a result of an antibody response to a wide range of antigens, for instance to antigens of infectious agents. Well-known examples of substances that have the property of binding to antibody-antigen complexes are complement 1q (C1q) and rheumatoid factor (RF).

C1q is one of the components of the complement system and is known specifically to bind to antibody-antigen complexes. C1q initiates the classical pathway of complement activation and mediates the solubilisation and clearance of immune complexes from circulation.

C1q is a heat-labile 11S protein which is composed of two non-identical and non-covalently bound subunits made up of three polypeptide chains. The native C1q has six distinct globular heads projecting from a stem-like tail region which contains collagen-like amino acid and carbohydrate sequences. It has been shown that C1q through the heads binds only to antibodies which are part of immune complexes, but essentially do not to monomeric antibodies.

RF is an antiglobulin antibody, i.e. an antibody binding to complexes of immunoglobulins, e.g. antibody-antigen complexes. RF is found in sera from persons suffering from autoimmune diseases such as rheumatoid arthritis but may also be produced experimentally by immunisation of animals with their own immunoglobulins in complexed form.

In WO 92/07267, fragments of C1q, synthetic peptides and methods for the detection or quantification of immune complexes present in a sample are disclosed. The method described comprises contacting the immune complexes with a fragment as claimed in this document whereby the immune complexes bind to the fragment, and detecting or quantitating the immune complexes. It has been suggested that this removal of immune complexes may be applied in the treatment or the diagnosis of various diseases.

From EP-A 9 198, a method is known for the detection and determination of a complement binding antibody, which method is characterised in that a binding partner (i.e. an antigen) to the complement binding antibody is immobilised, the immobilised antigen is reacted with the complement binding antibody and with serum complement, and the reaction product is reacted with a coupling product and a label. This method is only useful for antigens that are not adversely influenced by the immobilisation onto a solid support.

From US 4 062 935, US 4 138 213, US 4 143 124 and US 4 283 383, methods of analysing a biological fluid for the presence of antibodies, antigens or antibody-antigen complexes are known. The herein described methods are multi-step methods and they involve the reaction between antibody-antigen complexes and C1q or RF. Most of the described methods require the subsequent separation f th antigen, antibody or antibody-antigen complexes before the detection can be made. A quanti-

tative determination further requires knowledge of th amount of C1q, RF or antigen or antibody involved in the reaction. In the broadest aspect, the methods described in US 4 062 953 and US 4 138 213 are based on the classical complement reaction. In a further embodiment, the methods disclosed herein comprise as an essential feature the formation of agglomerates of the formed antibody-antigen complexes. In US 4 143 124 and US 4 283 383, a method for the detection of an antibody or an antigen is described, whereby an appropriate antigen or antibody is added to the sample, and subsequently brought into contact with C1q or RF. As described in the specification, this method is carried out as a competitive method, i.e. antibody-antigen complexes originally in the sample compete for the binding to C1q or RF with antibody-antigen complexes formed by adding the appropriate antigen or antibody which further may be labelled.

In DE 42 14 589, a method for the quantitative determination of substances present in a fluid is disclosed, whereby an immunosensor having C1q coated thereon as capture substance is brought in contact with a solution containing the antibody-antigen complexes to be determined, and whereby the immunosensor is brought in contact with a reagent that causes the dissociation of C1q and the complexes to be determined. In accordance with the description, two antibodies may be used in the detection of one antigen. At least one of the antibodies may be labelled. As described in Example 3, labelled IgM and IgG antibodies are used for detecting heat denatured alkaline phosphatase. By use of two antibodies, the sensitivity of the assay is improved as compared to the use of only one antibody (Example 2). The sole purpose of the described method is to improve assay performance.

A large number of immunological methods have been developed for the detection of various antigens or antibodies. Such methods have to be rapid, reliable and preferably inexpensive and easy-performed. Especially, in the screening of a large number of samples, e.g. donor blood, it is of utmost importance that screening assays can be performed rapidly, and, furthermore, it will often be advantageous to be able to simultaneously detect the presence of more than one antigen or antibody in a test sample. A standard procedure for the detection of more than one antigen or antibody or different classes of one antibody is to perform individual tests. This is e.g. the case when testing for HIV or Hepatitis C virus. Another example is when testing for IgG and IgM antibodies. The pr sence of IgG antibodies are traditionally d termined

using an indirect assay whereas IgM antibodies are traditionally detected using an antibody capture assay. In these assays, the antibody or antigen must be immobilised onto a solid support. Another standard procedure for the detection of more than one antigen or antibody or classes of one antibody comprises immobilisation of more than one antibody or antigen onto the same solid support. However, in such cases, competition between antibodies or antigens to be immobilised may be a disadvantage.

In WO 94/15193 and EP 408 542, such competition between antibodies or antigens during immobilisation has been overcome by use of a plurality of solid supports. The binding of the antigens or antibodies present in the test sample takes place between these antigens or antibodies and the corresponding immobilised antibodies or antigens. This method is only useful for antigens/antibodies that are not adversely influenced by the immobilisation onto a solid support.

DESCRIPTION OF THE INVENTION

The present invention relates to a method for the detection of different antibodies and/or antigens simultaneously. It has surprisingly been found that this can be achieved without loss of sensitivity or specificity. In the broadest aspect, the present invention relates to a method for the detection of more than one antibody and/or more than one class of antibodies or more than one antigen, or of one or more antibodies and/or one or more classes of antibodies and one or more antigens present in a test sample, which method comprises

(1) contacting said test sample with one or more selected antigens which are or will be identically or differently labelled and/or one or more selected antibodies which are or will be identically or differently labelled so as in solution to form antibody-antigen complexes of

(a) said selected antigens and antibodies present in said test sample, and/or

(b) said selected antibodies and antigens present in said test sample,

capturing the formed antibody-antigen complexes by means of a sub-

stance having ability to bind antibody-antigen compl xes, and which substance is or will be immobilised onto a solid support,

(2) observing or measuring the captured labelled antibody-antigen complexes on the solid support, and relating said observation or measurement to the presence of antigens and/or antibodies in said test sample.

This simultaneous detection can be accomplished without having to reduce the amount of the individual test antigens and/or antibodies in the test assay which may result in loss of sensitivity, since, in the present method, there is no competition between the individual test antibodies or antigens in contrast to traditional methods. This is an advantage as compared to indirect antibody assays wherein all individual test antigens must, in competition, be immobilised onto the solid support prior to the binding of the antibodies in the sample. This is also an advantage as compared to antigen sandwich assays wherein all individual test antibodies, in competition, must be immobilised onto the solid support prior of the binding to antigens in the sample. This is further advantageous as compared to antibody capture assays wherein both specific and non-specific antibodies, in competition, are captured prior to the binding to test antigens. In accordance with the present method, antibodies and/or antigens present in the sample react to form complexes in solution prior to immobilisation onto the solid support. Thus, the antibody-antigen reactions resemble *in vivo* conditions.

It has surprisingly been found that the positive-to-negative ratio (P/N ratio) is significantly higher using the method according to the invention as compared to P/N ratios of traditionally used indirect assays, cf. Example 3 below.

When screening large numbers of samples for antibodies and/or antigens for which the population display a low prevalence, it may be advantageous to separate those samples which are negative as a response to different antigens and/or antibodies from those samples which are positive. Thus, additional testing for individual antigens and/or antibodies is only necessary in those often few cases where a positive response was obtained by the screening.

Anoth r advantage of the present method is that it is possible, and economical feasible, to screen for antibodies and/or antigens for which the population shows ex-

tremely low prevalence since in such cases, an additional antigen or antibody may simply be included in the screening assay.

SPECIFIC DESCRIPTION

In the broadest aspect, the present invention relates to a method for the simultaneous detection of more than one antibody and/or class of antibodies or more than one antigen, or of one or more antibodies and/or classes of antibodies and one or more antigens present in a test sample, which method comprises

(1) contacting said test sample with one or more selected antigens which are or will be identically or differently labelled and/or one or more selected antibodies which are or will be identically or differently labelled so as in solution to form antibody-antigen complexes of

(a) said selected antigens and antibodies present in said test sample, and/or
(b) said selected antibodies and antigens present in said test sample,

capturing the formed antibody-antigen complexes by means of a substance having ability to bind antibody-antigen complexes, and which substance is or will be immobilised onto a solid support,

(2) observing or measuring the captured labelled antibody-antigen complexes on the solid support, and relating said observation or measurement to the presence of antigens and/or antibodies in said test sample.

In a further embodiment, the present invention relates to a method comprising

(1) contacting said test sample with one or more selected identically or differently labelled antigens and/or one or more selected identically or differently labelled antibodies so as in solution to form antibody-antigen complexes of

(a) said selected antigens and antibodi s present in said test sample,

and/or

(b) said selected antibodies and antigens present in said test sample,

capturing the formed antibody-antigen complexes by means of a substance having ability to bind antibody-antigen complexes, and which substance is or will be immobilised onto a solid support,

(2) observing or measuring the captured labelled antibody-antigen complexes on the solid support, and relating said observation or measurement to the presence of antigens and/or antibodies in said test sample.

In another aspect, the present invention relates to a method for the simultaneous detection of antibodies and/or classes of antibodies, wherein said antibodies to be detected are more than one antibody and/or belong to more than one class of antibodies. In another embodiment, the present invention relates to a simultaneous method for the detection of antigens, wherein said antigens to be detected are more than one antigen.

In a further aspect, the present invention relates to a method for the simultaneous detection of antibodies and/or classes of antibodies and antigens, wherein said antibodies and antigens to be detected are one or more antibodies and/or one or more classes of antibodies and one or more antigens.

The immobilisation onto the solid support of the substance having ability to bind antibody-antigen complexes may be achieved by immobilisation directly onto the solid support of the substance having ability to bind antibody-antigen complexes. Such immobilisation may be effected by covalent or non-covalent binding. Such covalent or non-covalent binding onto a solid support may be performed by methods and by use of agents well known in the art.

Alternatively, the immobilisation of the substance having ability to bind antibody-antigen complexes (which may be bound or not bound to the formed antibody-antigen complexes) may be achieved by means of a binding partner which binding partner is immobilised onto th solid support. The immobilisation may be effected using an antibody to the substance having ability to bind antibody-antigen complexes or using a

streptavidin or avidin coated solid support and a biotinylated substance having ability to bind antibody-antigen complexes. Coating of a solid support with streptavidin or avidin may be performed by coating directly with streptavidin or avidin, or by use of macromolecules as described in, e.g., EP 269 092 B1.

Examples of substances having ability to bind antibody-antigen complexes are C1q, RF, a portion or fragment thereof, and a peptide which is capable of binding to the formed antibody-antigen complexes. A preferred substance having ability to bind antibody-antigen complexes is C1q.

In accordance with the present invention, the test sample, the selected antigens and/or selected antibodies, and the substance having ability to bind antibody-antigen complexes may be brought into contact immediately without any preincubation. It is to be understood that the substance having ability to bind antibody-antigen complexes may already be present in the test sample as a naturally-occurring component of the sample, or it may be provided. The substance having ability to bind antibody-antigen complexes may be provided in immobilised form or not in immobilised form. The sample may also be brought into contact with the selected antigens and/or antibodies (preincubation), and thereafter be contacted with the optionally immobilised substance having ability to bind antibody-antigen complexes. It is an option to bring the test sample in contact with the optionally immobilised substance having ability to bind antibody-antigen complexes, before the selected antigens and/or antibodies are introduced. Likewise, it is an option firstly to bring the selected antigens and/or antibodies into contact with the optionally immobilised substance having ability to bind antibody-antigen complexes, before providing the test sample.

At hospitals and laboratories, heating of samples to be tested is often carried out as a standard procedure in order to eliminate the risk of transmission of various pathogens. In connection with the present method, such heating procedure may also be carried out so as to inactivate any substance having ability to bind antibody-antigen complexes such as C1q optionally present in the test sample. The heating procedure is carried out at a temperature and for a period of time sufficient to inactivate such substances such as at a temperature of 56°C for 30 minutes. C1q as well as RF may also be removed from the sample by adsorption to aggregated immunoglobulins.

Therefore, in a further aspect, the invention relat s to a method for the detection of more than one antibody and/or class of antibodies or more than one antigen, or of one or more antibodies and/or classes of antibodies and one or more antigens present in a test sample, which method further comprises pretreating the test sample under conditions by which any substance having ability to bind antibody-antigen complexes which substance optionally is present in said test sample, such as, e.g., C1q, is inactivated or removed.

Alternatively, any substance having ability to bind antibody-antigen complexes present in the sample may be used to capture the formed antibody-antigen complexes. In such case, additional substances having ability to bind antibody-antigen complexes may optionally be added. Thus, when C1q of the sample is used, additional C1q may optionally be added. When C1q of the sample is used without additional C1q, the stability of this substance should be taken into consideration.

As used herein, the term "selected antigens" is intended to comprise native, synthetic and recombinant proteins or peptides capable of forming antibody-antigen complexes with the antibody to be detected. In the present context, the term "synthetic proteins or peptides" comprises proteins or peptides which are modified naturally-occurring proteins or peptides or which are non-naturally-occurring proteins or peptides. The term "modified naturally-occurring proteins or peptides" is intended to comprise proteins or peptides with alterations such as deletions, additions or substitutions. Synthetic proteins or peptides may be synthesised *in vitro* or *in vivo*.

As used herein, the term "selected antibodies" is intended to comprise complement fixing monoclonal, polyclonal and recombinant antibodies capable of forming antibody-antigen complexes with the antigen to be detected.

As used herein, the term "different antibodies" is intended to comprise antibodies with different specificities and/or classes of antibodies with the same specificity.

The expression "which are or will be labelled" is intended to comprise both a directly and an indirectly labelling of the selected antibodies and the selected antigens. The selected antigens and/or antibodies may, if convenient, be labelled with identical or different labels. In some cases, it may be advantageous to label the antigens and/or

antibodies with different labels, allowing observation or measurement of the individual antigen or antibody associated with that particular label.

In the present context, the "substance having ability to bind antibody-antigen complexes" comprises any substance being able to bind to antibodies that are part of complexes formed between antibodies and antigens, but that essentially do not bind to non-complexed antibodies. Such substances may be isolated from humans or animals such as sheep, goats, rabbits or guinea pigs, or may be prepared synthetically. Such substances comprise C1q, a portion or fragment thereof, RF, a portion or fragment thereof as well as a peptide having said ability. Fragments of C1q and peptides prepared synthetically are described in WO 92/07267.

It is to be understood that the expression "a substance having ability to bind antibody-antigen complexes" is intended to include one or more substances, each having such binding ability. An example is C1q and a fragment thereof.

In the present context, the term "test sample" or "sample" is intended to include, but is not limited to, serum, saliva, tears, semen, urine, cerebrospinal fluid, milk, excretions, nasopharyngeal secretions (NPS), bronchial alveolar lavage (BAL), swabs, faecal samples, aspirates and the like. Such samples may be analysed immediately or stored under suitable conditions. The samples may be used undiluted (neat), diluted or concentrated.

As used herein, the term "binding partner" is a compound by which the substance having ability to bind antibody-antigen complexes is immobilised. The term is intended to comprise, but is not limited to, antibodies to the substance having ability to bind antibody-antigen complexes. An example of a useful binding partner may be rabbit anti-human C1q antibody, rabbit anti-guinea pig C1q antibody, streptavidin, and avidin. When streptavidin or avidin is used as binding partner, the substance having ability to bind antibody-antigen complexes must be biotinylated. Examples of monoclonal antibodies which are able to react with human C1q-containing complexes are described in WO 85/02261.

The expression "is or will be immobilised" is intended to encompass that the substance having ability to bind antibody-antigen complexes is immobilised prior to

contact with the test sample and th select d antigens and/or antibodies, as well as immobilisation after contact with th test sample. In this case, th substance having ability to bind antibody-antigen complexes must be immobilised by means of a binding partner.

The solid support capture system may take a wide variety of forms well known in the art, such as e.g. a plate, a microtiter plate having one or more wells, a microscope slide, a filter, a membrane, a tube, a dip stick, a strip, beads such as paramagnetic beads, beads made of polystyrene, polypropylene, polyethylene, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides and agaroses. When a filter, a membrane, a strip or beads is (are) used as the solid support, it (they) may, if conveniently be incorporated into a single-use device. Another attractive solid support is a multi-well microtiter plate which is very attractive, since such system is feasible for automatization of the analysis.

The label which is or will be attached to the selected antigen or selected antibody may suitably be an enzyme label, a fluorophore, a hapten, a radioisotope label, a peptide label, a coenzyme label, a dye, a donor or acceptor for electron transfer or a chemiluminiscent label.

Useful enzyme labels include, but are not limited to, peroxidases such as horseradish peroxidase (HRP), alkaline phosphatase (AP), alcohol dehydrogenases and glucose oxidase. Useful coenzyme labels include, but are not limited to, nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, flavin adenine dinucleotide and flavin adenine dinucleotide phosphate. Useful hapten labels include biotin, dinitrobenzoic acid and digoxigenin. Useful dye labels are, e.g., cyanine dyes such as Cy2, Cy3 and Cy5, methylene blue and colloidal gold.

As used herein, the term "fluorophore" is intended to mean a substance or a portion thereof which is capable of exhibiting fluorescence in a detectable range such as 5-(and 6)-carboxyfluorescein, 5-carboxyfluorescein, 6-carboxyfluorescein, fluorescein isothiocyanate (FITC), rhodamine, dansyl, umbeliferone, tetramethylrhodamine, cyanine dyes such as Cy2, Cy3 and Cy5, coumarin, R-phycoerythrin (RPE), allophycoerythrin, T xas Red and Princeston Red as well as conjugates of R-phycoerythrin and e.g. Cy5 and Texas Red.

Particularly useful labels are HRP, AP, biotin, digoxigenin, 5-(and 6)-carboxyfluorescein, FITC, streptavidin, avidin, dinitro benzoic acid, rhodamine, Cy5, R-phycoerythrin (RPE), RPE-Cy5, RPE-Texas Red and colloidal gold.

When the selected antibody or selected antigen is directly labelled, the label is coupled to such antibody or antigen. When the selected antibody or antigen is indirectly labelled, the label may, e.g., be introduced by using a labelled antibody or a fragment thereof which is bound to or is able to bind to the selected antibody or antigen.

The observing or measuring of the captured labelled antibody-antigen complexes may be performed using techniques generally known in the art. Such techniques include, i.a., the application of enzyme immunoassays (EIA), fluorescence immunoassays and chemiluminiscence immunoassays.

In one embodiment, the selected antigens or the selected antibodies used in the present method are antigens derived from or antibodies against virus, bacteria, fungi, parasites, toxins or allergens.

In accordance with the present invention, selected antigens may be derived from or selected antibodies may be raised against virus such as RSV (Respiratory Syncytial Virus), Astrovirus, Adenovirus, Rotavirus, SRSV (small round structured viruses), Parvovirus B19, Measles, Mumps, Rubella, CMV (Cytomegalovirus), EBV (Epstein-Barr Virus), Herpes Simplex Virus (HSV), hepatitis viruses, HIV (human immunodeficiency viruses), HTLV (human T cell lymphotropic viruses), Influenza Virus, Parainfluenza Virus, Papillomavirus, Enterovirus and the like.

In accordance with the present invention, selected antigens may be derived from or selected antibodies may be raised against bacteria such as Chlamydia spp., Borrelia spp., Rickettsia spp., Leptospira spp., Yersinia spp., Treponema spp., Mycobacterium spp., Mycoplasma spp., Neisseria spp., Streptococcus spp., Helicobacter spp., Staphylococcus spp., Salmonella spp., Legionella spp. and the like.

In accordance with the present invention, selected antigens may be derived from or selected antibodies may be raised against fungi such as Candida spp., Cryptococcus

spp. and th like.

In accordance with the present invention, selected antigens may be derived from or selected antibodies may be raised against parasites such as Toxoplasma spp. and the like.

In accordance with the present invention, selected antigens may be derived from or selected antibodies may be raised against toxins such as Clostridium toxins, Pasteurella multocida toxin and the like.

In another aspect, the present invention relates to the use of the method described herein for the diagnosis of or screening for disorders or diseases caused by virus, bacteria, fungi, parasites, toxins or allergens. The method of the invention may be used to detect the presence of Astrovirus, Adenovirus and Rotavirus in faecal samples by applying the relevant selected antibodies. The method of the invention may further be used to detect classes of antibodies to Borrelia burgdorferi, or to Treponema pallidum by applying the respective selected antigen. The method of the invention may also be used to detect antibodies to HIV-I, HIV-II, HTLV-I, HTLV-II and Hepatitis C virus by applying five selected antigens. The method of the invention may be used to detect Hepatitis B virus and antibodies to Hepatitis C virus by applying the relevant selected antibody and antigen.

A kit for performing the method described above also constitutes part of the present invention, which kit comprises one or more selected antigens and/or one or more selected antibodies, and one or more reagents for labelling the antibody-antigen complexes or constituents of such complexes, and a solid support capture system with a substance having ability to bind antibody-antigen complexes immobilised thereon, or a solid support and one or more reagents for such immobilisation.

In another embodiment, such kit comprises one or more selected labelled antigens and/or one or more selected labelled antibodies, and a solid support capture system with a substance having ability to bind antibody-antigen complexes immobilised thereon, or a solid support and reagents for such immobilisation. The substance having ability to bind antibody-antigen complexes is preferably C1q or a fragment thereof. C1q may suitably be immobilised onto the solid support by means of a

binding partner, which binding partner may preferably be an antibody against C1q such as rabbit anti-human C1q antibody.

The kit may further comprise a detection system for visualising any labels such as enzyme labels that may not be observed directly. The detection system may suitably comprise enzyme substrates as well as reagents for stopping the enzyme reaction.

In another embodiment, the kit may further comprise a signal amplifying system.

In some cases, it may be convenient to label the different selected antigens and/or antibodies with different labels (non-identical labels), thereby relating different observed signals to the presence of particular antigens and/or antibodies.

For ELISA tests, AP and HRP may suitably be used. The enzyme activity may then be visualised using substrates consecutively, whereby the solid support capture system is separated from the first substrate before the second substrate is introduced. The activity may then be observed at different wavelengths. More than two labels may be used.

For observation using a fluorescence microscope, FITC and RPE may suitably be used.

Flow cytometry is another technique which is very suitable for analysing samples and which is, furthermore, suitable for automated analysis. The flow cytometer may utilise a single light source or more than one separate light source may be used to excite each different type of fluorochrome or immunofluorescent labels. Useful labels are FITC, RPE, RPE-Cy5, RPE-Texas Red and allophycocyanine.

Beads for use in flow cytometry may preferably be spherical particles with a diameter of from 1 to 30 μm, preferably from 1 to 20 μm, more preferably from 2 to 15 μm and most preferably from 2 to 10 μm. Particles having a diameter of from 5 to 10 μm are particular convenient since this is approximately the size of biological cells, and, thus, the parameters of the flow cytometer need not to be changed. The size of th particles should, of cause, be chosen so as to be suitable for the desired assay performance.

The invention is further illustrated by the following examples.

EXAMPLE 1

Simultaneous detection of two classes of antibodies (IgM and IgG) to Borrelia burgdorferi in human serum

EXAMPLE 1A

43 serum samples from patients suffering from Lyme Borreliosis as well as 144 serum samples from presumed healthy blood donors from an endemic area were tested as described below for the presence of antibodies to *B. burgdorferi*. The results obtained by use of the method according to the invention for the detection of IgM and IgG antibodies to *B. burgdorferi* are compared with the results obtained by use of commercially available test kits for the separate detection of IgG and IgM antibodies to *B. burgdorferi* (IDEIA™ *Borrelia burgdorferi* IgG and IDEIA™ *Borrelia burgdorferi* IgM, DAKO).

Microwells (MaxiSorp®, NUNC) were precoated with 100 µl, 5 µg/ml rabbit anti-human C1q antibody (DAKO) and were stored at 4°C prior to use.

The serum samples were diluted 1 to 100 in tris buffered saline with pH 7.2 (TBS). In test tubes, volumes of 150 µl of the diluted sample were mixed with volumes of 150 µl flagellum conjugate (biotinylated *B. burgdorferi* flagellum complexed with streptavidin labelled with HRP) which are components of the IDEIA™ *Borrelia burgdorferi* IgM test kit) and were preincubated 1 hour at room temperature. During this preincubation, 100 µl, 1 µg/ml human C1q (Sigma) was added to each of the microwells which had been precoated with rabbit anti-human C1q antibody, and the microwells were incubated for 1 hour at room temperature on an orbital shaker. Then, the microwells were washed four times with 300 µl of TBS. To the microwells, 100 µl of each sample mixture (from the test tubes) was added in duplicate, and the microwells were incubated for 1 hour at room temperature on an orbital shaker.

The microwells were washed four times with 300 µl TBS, and 100 µl TMB (tetramethylbenzidine) substrate was added to the wells. Bound HRP was visualised by

th development of a blue colour. The enzyme reaction was stopped after 10 minutes by addition of 100 µl 0.5 M sulphuric acid which changed the colour from blue to yellow. The intensity of the colour corresponded to the amount of the captured labelled antibody-antigen complexes. The colour intensity was determined spectrophotometrically as OD-values read at 450 nm. The cut-off was defined to be OD = 0.200.

The obtained results indicated in Table 1 and 2 are the number of positive and negative responses, respectively, for the 43 serum samples from patients suffering from Lyme Borreliosis when tested with the IDEIA™ *Borrelia burgdorferi* IgM and IDEIA™ *Borrelia burgdorferi* IgG kits. In the tables, the corresponding results obtained when the same serum samples were tested by use of the method according to the invention are given. In Table 3, the results from Table 1 and 2 are summarised. In Table 3, the number of serum samples indicated as being positive corresponds to the number of serum samples containing either IgM or IgG antibodies to *B. burgdorferi* as well as both IgM and IgG antibodies to *B. burgdorferi*.

TABLE 1

		MAI	MAI	
		Pos	Neg	Total
IDEIA™ IgM	Pos	26	0	26
IDEIA™ IgM	Neg	14*	3	17
	Total	40	3	43

IDEIA™ IgM: IDEIA™ *Borrelia burgdorferi* IgM

MAI: Method according to the invention for detection of IgM and IgG antibodies to *B. burgdorferi*

Total: Total number of samples

* All 14 samples were positive in the IDEIA™ *Borrelia burgdorferi* IgG kit

TABLE 2

		MAI Pos	MAI Neg	Total
IDEIA™ IgG	Pos	34	0	34
IDEIA™ IgG	Neg	6*	3	9
	Total	40	3	43

IDEIA™ IgG IDEIA™ *Borrelia burgdorferi* IgG

Other abbreviations as above

* All 6 samples were positive in the IDEIA™ *Borrelia burgdorferi* IgM kit

TABLE 3

		MAI Pos	MAI Neg	Total
IDEIA™ IgM/IgG	Pos	40	0	40
IDEIA™ IgM/IgG	Neg	0	3	3
	Total	40	3	43

Abbreviations as above

From Table 1, it can be seen that all serum samples which were positive with respect to IgM antibodies to *B. burgdorferi* were also positive when tested by use of the present method. 14 of 17 IgM negative serum samples were positive when tested by use of the method according to the invention. This observation is due to the presence in these serum samples of specific IgG antibodies to *B. burgdorferi* which are not detected by use of the IDEIA™ *Borrelia burgdorferi* IgM kit but which are detected by use of the present method. The presence of IgG antibodies in these 14 samples was confirmed by the IDEIA™ IgG kit.

From the Table 2, it can be seen that all serum samples which were positive with respect to IgG antibodies to *B. burgdorferi* were also positive when tested by use of the present method. 6 of 9 IgG negative serum samples were positive when tested by use of the present method. This observation is due to the presence in these serum samples of specific IgM antibodies to *B. burgdorferi* which are not detected by use of the IDEIA™ *Borrelia burgdorferi* IgG kit but which are detected by use of the method according to the invention. The presence of IgM antibodies in these 6 sam-

ples was confirmed by the IDEIA™ IgM kit.

Table 3 summarises the results from Table 1 and 2. It can be seen that the diagnostic sensitivity of the present method was similar to the diagnostic sensitivity of the combined use of the IDEIA™ *Borrelia burgdorferi* IgM and IgG kits since all positive serum samples (i.e. serum samples containing either IgM or IgG antibodies as well as serum samples containing both IgM and IgG antibodies when tested in the commercially available kits) were positive when tested by use of the present method.

The obtained results indicated in Table 4 and 5 show the number of positive or negative responses for the 144 serum samples from blood donors (with a low prevalence of antibodies to *B. burgdorferi*) when tested with the IDEIA™ IgM and IgG kit. In the tables, the results obtained when the same serum samples were tested by use of the present method are shown. In Table 6, the results from Table 4 and 5 are summarised.

TABLE 4

		MAI Pos	MAI Neg	Total
IDEIA™ IgM	Pos	3	0	3
IDEIA™ IgM	Neg	2*	139	141
	Total	5	139	144

Abbreviations as above

* Both samples were positive in the IDEIA™ *Borrelia burgdorferi* IgG kit

TABLE 5

		MAI Pos	MAI Neg	Total
IDEIA™ IgG	Pos	2	0	2
IDEIA™ IgG	Neg	3*	139	142
	Total	5	139	144

Abbreviations as above

* All 3 samples were positive in the IDEIA™ *Borrelia burgdorferi* IgM kit

TABLE 6

		MAI Pos	MAI Neg	Total
IDEIA™ IgM/IgG	Pos	5	0	5
IDEIA™ IgM/IgG	Neg	0	139	139
	Total	5	139	144

Abbreviations as above

From Table 4, it can be seen that 3 of 144 serum samples contained IgM antibodies to *Borrelia burgdorferi*, i.e. were positive, when tested with the IDEIA™ IgM kit. These samples were also positive when tested by use of the present method. 2 of 141 IgM negative serum samples were positive when tested by the present method. This observation is due to the presence in these serum samples of specific IgG antibodies to *B. burgdorferi* which are not detected by use of the IDEIA™ IgG kit but which are detected by the present method. The presence of IgG antibodies in these 2 samples was confirmed by the IDEIA™ IgG kit.

From Table 5, it can be seen that 2 of 144 serum samples contained IgG antibodies to *B. burgdorferi*. These 2 serum samples were also positive when tested by use of the method according to the invention. 3 of 142 IgG negative serum samples were also positive when tested by use of the present method. This observation is due to the presence in these serum samples of specific IgM antibodies to *B. burgdorferi* which are not detected by the IDEIA™ IgG kit but which are detected by use of the present method. The presence of IgM antibodies in these 3 samples was confirmed by the IDEIA™ IgM kit.

It can be seen from Tables 4, 5 and 6, that the diagnostic specificity of the present method was similar to the diagnostic specificity of the combined use of the IDEIA™ *Borrelia burgdorferi* IgM and IgG kits since all negative serum samples (i.e. serum samples containing neither IgM nor IgG antibodies when tested by the IDEIA™ IgM and IgG kits) were negative when tested by the method according to the invention.

The obtained r sults clearly show the advantages of the method of the invention. Only one test is required with th present method whereas two s parate tests are

required when the commercially available kits are used.

EXAMPLE 1B

In another experiment, each microwells were firstly coated with 100 µl, 5 µg/ml rabbit anti-human C1q antibody, washed 4 times in 300 µl TBS, and subsequently coating with 100 µl, 2.5 µg/ml human C1q (double-coating) and were stored at 4°C prior to use. Thus, no preincubation with human C1q is necessary. The test results so obtained were similar to the above-described.

EXAMPLE 1C

In another experiment, serum samples diluted 1:100 in TBS were incubated for two hours with flagellum conjugate (biotinylated *B. burgdorferi* flagellum complexed with streptavidin labelled with horseradish peroxidase (HRP)) directly in microwells double-coated with a rabbit anti-human C1q antibody-human C1q. Thus, preincubation between the sample and the flagellum is not needed, and the procedure is accordingly simplified. The test results so obtained were similar to the above-described.

EXAMPLE 2

Simultaneous detection of antibodies to B. burgdorferi and to Parvovirus B19 in human serum

Serum samples from three patients suffering from Lyme Borreliosis (samples denoted numbers 9, 12 and 15, respectively) and serum samples from five healthy blood donors (samples denoted numbers 343, 348, 318, 347 and 302, respectively) were tested using the method according to the invention. The detection of antibodies to *B. burgdorferi* and/or to Parvovirus B19 was carried out as described below.

The serum samples were selected using the following commercially available test kits: IDEIA™ Parvovirus B19 IgG, IDEIA™ Parvovirus B19 IgM, IDEIA™ *Borrelia burgdorferi* IgG and IDEIA™ *Borrelia burgdorferi* IgM kits (all kits from DAKO). The samples were chosen so as to represent all combinations of IgG antibodies to *B. burgdorferi* and Parvovirus B19, respectively, i.e. both *B. burgdorferi* and Parvovirus B19 positive or negative, *B. burgdorferi* positive and Parvovirus B19 negativ , and *B. burgdorferi* negativ and Parvovirus B19 positive. The samples were further selected so as to be negative with respect to IgM antibodies to both *B. burgdorferi* and Par-

vovirus B19.

Microwells (MaxiSorp®, NUNC) were precoated with human C1q (100 µl, 1 µg/ml human C1q) and were stored at 4°C prior to use.

Serum samples were diluted 1:100 in tris buffered saline with pH 7.2 (TBS) and were heated at 56°C for 30 minutes in order to inactivate any C1q present in the serum samples. In test tubes, volumes of diluted sample were mixed with an equal volume of diluted biotinylated recombinant Parvovirus B19 capsids, an equal volume of biotinylated *Borrelia burgdorferi* flagellum, or an equal volume of a mixture of biotinylated recombinant Parvovirus B19 capsids and biotinylated *Borrelia burgdorferi* flagellum, respectively. Biotinylated recombinant Parvovirus B19 capsids and biotinylated *B. burgdorferi* flagellum were components of the IDEIA™ Parvovirus B19 IgM kit and the IDEIA™ *Borrelia burgdorferi* IgM kit, respectively. The sample mixtures were preincubated one hour at room temperature. After incubation, 100 µl of each sample mixture was added in duplicate to microwells coated with human C1q. The microwells were incubated for one hour at room temperature on an orbital shaker.

Washing and detection was carried out as described in Example 1. The cut-off was defined to be OD = 0.100.

Table 7 shows the results obtained by use of the IDEIA™ kits as well as the results obtained by the present method according to the invention for detection of IgM and/or IgG antibodies to Parvovirus B19, to *Borrelia burgdorferi* and simultaneously to Parvovirus B19 and *B. burgdorferi*, respectively.

TABLE 7

Sample number	Commercially available test kits				Method of the invention		
	IDEIA™ Parvo IgG	IDEIA™ Parvo IgM	IDEIA™ Borrelia IgG	IDEIA™ Borrelia IgM	MAI Parvo	MAI Borrelia	MAI Parvo/ Borrelia
302	+	-	-	-	+	-	+
318	+	-	-	-	+	-	+
343	-	-	-	-	-	-	-
347	+	-	-	-	+	-	+
348	-	-	-	-	-	-	-
9	-	-	+	-	-	+	+
12	+	-	+	-	+	+	+
15	+	-	+	-	+	+	+

IDEIA™ Parvo IgG: IDEIA™ Parvovirus B19 IgG

IDEIA™ Parvo IgM: IDEIA™ Parvovirus B19 IgM

IDEIA™ Borrelia IgG: IDEIA™ *Borrelia burgdorferi* IgG

IDEIA™ Borrelia IgM: IDEIA™ *Borrelia burgdorferi* IgM

MAI Parvo: Method according to the invention for the detection of antibodies to Parvovirus B19

MAI Borrelia: Method according to the invention for the detection of antibodies to *B. burgdorferi*

MAI Parvo/Borrelia: Method according to the invention for the simultaneous detection of antibodies to Parvovirus B19 and *B. burgdorferi*

\+ Positive response, OD > 0.100

\- Negative response, OD ≤ 0.100

As it appears from Table 7, the serum samples from healthy donors (serum samples with numbers 302, 318, 343, 347 and 348) were negative with respect to IgM and/or IgG antibodies to *B. burgdorferi* when tested by the present method. When tested by the present method for the detection of antibodies to Parvovirus B19, serum samples 302, 318, 347, 12 and 15 were found positive. These results were confirmed by the IDEIA™ kits. From Table 7, it can further be seen that the result obtained by th simultaneous detection of IgM and/or IgG antibodies to both *B. burgdorferi* and Parvovirus B19 are in conformity with the findings by the separate tests.

EXAMPLE 3

Comparison of the P/N ratios obtained with the present method and with an indirect assay for the detection of antibodies to B. burgdorferi

A serum sample containing only IgG antibodies to *B. burgdorferi* was selected for this experiment and was diluted in a serum sample that did not contain IgM nor IgG antibodies to *B. burgdorferi* in order to imitate serum samples containing different concentrations of specific IgG antibodies. These serum samples were analysed by the IDEIA™ *Borrelia burgdorferi* IgG kit and by use of the method according to the invention for the detection of antibodies to *B. burgdorferi*. The detection was carried out as described in Example 1A.

The results are shown in Table 8.

TABLE 8

Dilution, 2-fold serial	OD-values		P/N	
	IDEIA™ IgG	MAI	IDEIA™ IgG	MAI
1 ×	2251	2897	32	38
2 ×	1971	3200	28	42
4 ×	1538	3318	22	44
8 ×	1177	3370	17	44
16 ×	692	2445	9.7	32
32 ×	367	573	5.2	7.5
64 ×	159	108	2.2	1.4
128 ×	69	71	1.0	0.9
256 ×	71	76	1.0	1.0

IDEIA™ IgG: IDEIA™ *Borrelia burgdorferi* IgG

MAI: Method according to the invention for the detection of IgM and IgG antibodies to *B. burgdorferi*

In Tabl 8, the P/N ratios obtained with the indirect IDEIA™ *Borrelia burgdorferi* IgG are shown. The OD-values obtained by use of the method according to the invention

for the detection of IgM and IgG antibodies are also shown. The P/N ratio is defined as the OD-values of each sample divided by the OD-value of the sample representing a dilution of 256×. The cut-off of the commercially available IgG kit is between dilutions of 32× and 64× (not shown).

The differences in the P/N ratios between positive samples (dilution ≤32×) and negative samples (dilution ≥64) are much more significant when the method according to the invention is used. This is especially observed for samples with a medium to low content of specific IgG antibodies (dilution 4× to 16×). Thus, a much better discrimination between positive and negative samples is obtained with the method according to the invention.

EXAMPLE 4

Use of C1q of serum samples as the substance with ability to bind labelled antibody-antigen complexes for simultaneous detection of IgG and IgM antibodies to Treponema pallidum

C1q present in human serum samples was used as substance with ability to bind antibody-antigen complexes. Rabbit anti-human C1q antibody was used as binding partner for immobilising C1q onto the solid support.

In microwells (precoated with 100 µl, 0.05 µg/ml rabbit anti-human C1q antibody), 5 µl of the serum samples was mixed with 50 µl TBS and 50 µl Treponema flagellum conjugate (biotinylated Treponema flagellum (Scand. J. Immunol. 15, 341-348 (1982)) complexed with HRP labelled streptavidin) and incubated two hours at room temperature. As reference, mixtures were also incubated in double-coated microwells (100 µl, 0.05 µg/ml rabbit anti C1q antibody and 100 µl, 1 µg/ml C1q as described in Example 1A).

Visualisation was performed as described in Example 1 with the exception that the enzyme reaction was stopped after 30 minutes. The cut-off was defined to be OD = 0.350.

Th results are given in Table 9 below. In the table, MIA - are the results obtained with microwells coated only with rabbit anti-human C1q antibody, and MIA + are the results obtained with the rabbit anti-human C1q antibody-human C1q double-coated

microwells (for reference).

TABLE 9

		MAI - C1q	MAI - C1q	
		Pos	Neg	Total
MAI + C1q	Pos	23	0	23
MAI + C1q	Neg	2	35	37
	Total	25	35	60

Pos	Positive samples
Neg	Negative samples
MAI	Method according to the invention for the detection of antibodies to *T. pallidum*
- C1q	Microwells coated with rabbit anti-human C1q antibody
+ C1q	Microwells double-coated with rabbit anti-human C1q antibody-human C1q

As can be seen from the table, two of the samples tested by use of the microwells only coated with rabbit anti-human C1q were not in conformity with the reference test. This should be further investigated. It is contemplated that addition of extra C1q to these samples will give a result being in conformity with the reference test.

EXAMPLE 5

One-step immunofluorescence method for the simultaneous detection of antibodies against Borrelia burgdorferi and Treponema pallidum in human serum

The following serum samples were tested by the present method: One serum sample being positive with respect to antibodies against *Borrelia burgdorferi* (Borrelia positive) and negative with respect to antibodies against *Treponema pallidum* (Syphilis negative), one serum sample being Borrelia negative and Syphilis positive, and one serum sample being Borrelia negative and Syphilis negative. The serum samples were confirmed positive or negative with the commercially available kit IDEIA™ *Borrelia burgdorferi* IgG (DAKO), and with a prototype Syphilis IgG (made by DAKO).

Sulphate polystyrene latex beads (0.1% w/v) having a diameter of 5 µm (Interfacial

Dynamics Corporation) were coated with human C1q (5 µg/ml C1q in PBS two hours at room temperature). C1q coated beads were washed three times in TBS and were mixed in a tube with serum sample diluted 1:100 in TBS, and FITC labelled antigens (biotinylated Borrelia burgdorferi flagellum mixed with FITC labelled streptavidin (DAKO) or biotinylated Treponema flagellum mixed with FITC labelled streptavidin (DAKO)). The mixture was incubated without further treatment in the dark for two hours at room temperature. The beads were then analysed by flow cytometry (Becton Dickinson FACScan flow cytometer).

The obtained results are shown in Table 10 below. The results are given as mean fluorescence intensity.

TABLE 10

Antigen / Sample	MAI Borrelia/FITC	MAI Syphilis/FITC	MAI Borrelia/FITC MAI Syphilis/FITC
Borrelia positive Syphilis negative	10^2	2×10^1	10^2
Borrelia negative Syphilis positive	2×10^1	10^2	10^2
Borrelia negative Syphilis negative	2×10^1	2×10^1	2×10^1

MAI Borrelia/FITC — Method according to the invention for the detection of antibodies to *B. burgdorferi*

MAI Syphilis/FITC — Method according to the invention for the detection of antibodies to *T. pallidum*

MAI Borrelia/FITC MAI Syphilis/FITC — Method according to the invention for the simultaneous detection of antibodies to *B. burgdorferi* and *T. pallidum*

As it appears from the table, neither a positive signal nor a negative signal is influenced by the presence of two antigens as compared to the presence of only one antigen. These results indicate that the method according to the invention can advantageously be used to detect antibodies against two different antigens without reduction in sensitivity or specificity.

An important feature of this assay format is that the fluorescence intensity is meas-

ured directly on the solid support. As it appears from the above-d scribed, no separation step is necessary before analysis of the captured FITC labelled antibody-antigen complexes.

EXAMPLE 6

Simultaneous detection of several different serum proteins

Microwells were double-coated with rabbit anti-human C1q antibody-human C1q as described in Example 1B and stored at 4°C prior to use.

Biotinylated rabbit anti-human IgA antibodies (DAKO), biotinylated rabbit anti-human IgM antibodies (DAKO) and biotinylated rabbit anti-human IgG antibodies (DAKO), each diluted 1:10 in TBS, were each preincubated for one hour in test tubes with streptavidin labelled with HRP (DAKO) diluted 1:5000 in buffer TBS.

The HRP labelled antibodies were used separately or in combination (see Table 11). The labelled antibodies were mixed 1:1 with 2.5 µg/ml human IgA (DAKO), 5 µg/ml human IgM (DAKO) or 2.5 µg/ml human IgG (DAKO) either separately or in combination. All mixtures were additionally tested against buffer as a control. Incubation was performed for one hour at room temperature.

Subsequently, 100 µl of each mixture was transferred to the rabbit anti-human C1q antibody-human C1q double-coated microwells, incubated for 1 hour at room temperature on an orbital shaker, and washed 4 times with a TBS buffer. Visualisation was carried out as described in Example 1.

TABLE 11

Antibody / Antigen	Anti IgA/ HRP	Anti IgM/ HRP	Anti IgG/ HRP	Anti IgA/M/ HRP	Anti IgA/G/ HRP	Anti IgM/G/ HRP	Anti IgA/M/G/ HRP
IgA	+	-	-	+	+	-	+
IgM	-	+	-	+	-	+	+
IgG	-	-	+	-	+	+	+
IgA/M	+	+	-	+	+	+	+
IgA/G	+	-	+	+	+	+	+
IgM/G	-	+	+	+	+	+	+
IgA/M/G	+	+	+	+	+	+	+
Buffer	-	-	-	-	-	-	-

\+ The signal is positive if $OD \geq 3 \times OD_{Buffer}$

\- The signal is negative if $OD < 3 \times OD_{Buffer}$

Anti Abbreviation for rabbit anti-human antibodies

CLAIMS

1. Method for the detection of more than one antibody and/or class of antibodies or more than one antigen, or of one or more antibodies and/or classes of antibodies and one or more antigens present in a test sample, which method comprises

(1) contacting said test sample with one or more selected antigens which are or will be identically or differently labelled and/or one or more selected antibodies which are or will be identically or differently labelled so as in solution to form antibody-antigen complexes of

(a) said selected antigens and antibodies present in said test sample, and/or

(b) said selected antibodies and antigens present in said test sample,

capturing the formed antibody-antigen complexes by means of a substance having ability to bind antibody-antigen complexes, and which substance is or will be immobilised onto a solid support,

(2) observing or measuring the captured labelled antibody-antigen complexes on the solid support, and relating said observation or measurement to the presence of antigens and/or antibodies in said test sample.

2. Method according to claim 1, which method comprises

(1) contacting said test sample with one or more selected identically or differently labelled antigens and/or one or more selected identically or differently labelled antibodies so as in solution to form antibody-antigen complexes of

(a) said selected antigens and antibodies present in said test sample, and/or

(b) said selected antibodies and antigens present in said test sample,

capturing the formed antibody-antigen compl xes by means of a

substance having ability to bind antibody-antigen complexes, and which substance is or will be immobilised onto a solid support,

(2) observing or measuring the captured labelled antibody-antigen complexes on the solid support, and relating said observation or measurement to the presence of antigens and/or antibodies in said test sample.

3. Method according to claim 1 or 2, wherein said antibodies to be detected are more than one antibody and/or more than one class of antibodies.

4. Method according to claim 1 or 2, wherein said antigens to be detected are more than one antigen.

5. Method according to any one of claims 1-4, wherein the immobilisation of the substance having ability to bind antibody-antigen complexes is achieved either by immobilisation of said substance directly to the solid support or by means of a binding partner.

6. Method according to claim 5, wherein the binding partner is an antibody to the substance having ability to bind antibody-antigen complexes.

7. Method according to any one of claims 1-6, which method further comprises pretreating the test sample under conditions by which any substance having ability to bind antibody-antigen complexes optionally present in said test sample is inactivated or removed.

8. Method according to any one of claims 1-7, wherein the substance having ability to bind antibody-antigen complexes is C1q, a portion or fragment thereof, RF, a portion or fragment thereof, or a peptide which are capable of binding to the formed antibody-antigen complexes.

9. Method according to claim 8, wherein the substance having ability to bind antibody-antigen complexes is C1q.

10. Method according to any one of claims 1-9, wherein the selected antigens and/or

selected antibodies are antigens derived from or antibodies against virus, bacteria, fungi, parasites, toxins or allergens.

11. Method according to any one of claims 1-10, wherein the selected antibodies and/or selected antigens are labelled with horseradish peroxidase (HRP), alkaline phosphatase (AP), biotin, 5-(and 6)-carboxyfluorescein, fluorescein isothiocyanate (FITC), digoxigenin, dinitro benzoic acid, rhodamine, Cy5, R-phycoerythrin (RPE), RPE-Cy5, RPE-Texas Red or colloidal gold.

12. Method according to any one of claims 1-11, wherein said observing or measuring of the captured labelled antibody-antigen complexes are performed by enzyme immunoassays (EIA), fluorescence immunoassays and chemiluminiscence immunoassays.

13. Use of the method according to any one of claims 1-12 for the diagnosis of or screening for disorders or diseases caused by viruses, bacteria, fungi, parasites, toxins or allergens.

14. Use of the method according to claim 13, for the diagnosis of diseases caused by Astrovirus, Adenovirus and Rotavirus, or by HIV viruses, HTLV viruses and Hepatitis C virus, or by Hepatitis B virus and Hepatitis C virus.

15. Kit for performing the method according to any one of claims 1-12 for the detection of more than one antibody and/or more than one class of antibodies or more than one antigen, or of one or more antibodies and/or classes of antibodies and one or more antigens, which kit comprises one or more selected antigens and/or one or more selected antibodies, and one or more reagents for labelling the anti-body-antigen complexes or constituents thereof, and a solid support capture system with a substance having ability to bind antibody-antigen complexes immobilised thereon, or a solid support and one or more reagents for such immobilisation.

16. Kit according to claim 15, which kit comprises one or more selected labelled antigens and/or one or more selected labelled antibodies, and a solid support capture system with a substance having ability to bind antibody-antigen complexes immobilised thereon, or a solid support and reagents for such immobilisation.

17. Kit according to claim 15 or 16, which kit further comprises a detection system.

18. Kit according to any one of claims 15-17, wherein the substance having ability to bind antibody-antigen complexes is immobilised C1q.

INTERNATIONAL SEARCH REPORT

Interr•tional Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/537

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,4 283 383 (MASSON PIERRE L ET AL) 11 August 1981 cited in the application see claim 10	1-18
X	DE,A,42 14 589 (IMTEC IMMUNDIAGNOSTIKA GMBH) 11 November 1993 cited in the application see claims 5-7	1-18
X	WO,A,92 07267 (UNIV NORTHWESTERN) 30 April 1992 cited in the application see claims 4-6,17-19	1-18
X	WO,A,94 17410 (IMRE CORP ;BALINT JAMES P (US)) 4 August 1994 see the whole document	1-18

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 November 1996	09. 12. 96

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INTERNATIONAL SEARCH REPORT

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